Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

PROPOSAL NARRATIVE

(Max. ~~of 3 pages including figures/tables but excluding budget page, 1" margins, 12-point font. See Sec.~~XII of RFP for more details.)

Proposals not complying with format guidelines will not be considered.

I. Project Title: Effects of tick parasitism on aggressive behavior in male western fence lizards: geographic differences in self-assessment capacity?

II. Abstract: Although it is well known that parasites can produce substantial changes in the behavior of their hosts, few studies have addressed how individuals of the same host species from different locations respond behaviorally to a common parasite. We will use the ubiquitous and intensely territorial western fence lizard to address questions about the evolution of behavioral traits in response to tick infestation. There is a natural east-west gradient in tick abundance in SLO County, such that lizards in the west are subject to tick infestation whereas lizards in the east are not. Male lizards from both locations will be brought to Cal Poly, where half will be infested with the western blacklegged tick and undergo staged territorial disputes with size-matched, tick-free rivals. We predict that infested lizards from areas experiencing chronic tick infestation (i.e., western areas) will invest less in confronting rivals, because they have evolved to accurately downgrade their assessment of their ability to maintain territories. We predict that, in contrast, infested individuals from parasite naïve areas (i.e., eastern areas) will invest heavily in contests despite the fact that their capacity to win is reduced by tick parasitism.

III. Introduction: Blacklegged ticks can only survive in habitats with water[9,10]. Along the central coast, western blacklegged ticks (*Ixodes pacificus*) are abundant in western parts of San Luis Obispo County, but are absent inland. The western fence lizard (*Sceloporus occidentalis*) is abundant throughout SLO County, and is commonly parasitized by tick larvae and nymphs in the western part of its range[7,8,9]. *Sceloporus* males maintain territories through displays of their blue ventral surface (characteristic "push-up" displays), chasing and biting. In addition to physical attributes such as size and coloration, physiological characteristics, such as sprint speed, influence the capacity of individual lizards to win territorial conflicts[1]. Previous work has shown that heavy infection with *Plasmodium mexicanum* (lizard malaria) is correlated with anemia and reduced aerobic capacity that may hinder escape from predators as well as influence intrasexual conflict outcomes[2,11]. Additionally, blood feeding ectoparasites such as ticks can also induce anemia , reducing hematocrit by 10% in lizards[3,4]. Because both malaria and tick infestation can lead to anemia, and because malaria is known to reduce male fighting capacity to hold territories, tick infestation alone may similarly impede success of host lizards to win competitions and maintain territories. Animals face incentives to self-assess their abilities to avoid over-investment in conflicts they are unlikely to win[5]. Because parasites can impose severe physiological handicaps on their hosts, individuals in locales that have coevolved with parasites should develop the capacity to assess parasite burden when determining how much energy to invest in a conflict[12,14,15]. Individuals in coastal *S. occidentalis* populations should possess the capacity to assess parasite burden, and therefore reduce their investment in conflicts when infested. Inland populations should not alter their level of aggression, even though a parasitized inland male is just as unlikely to eventually win an otherwise evenly

matched contest as his coastal counterpart.

The impact of tick ectoparasitism alone as a mediating influence on competitive behavior in reptiles is poorly understood. Even less well understood is how behavior varies as a function of coevolution with parasites, both for *Sceloporus* lizards in particular and for animals generally. Through this research, we will contribute to the body of knowledge on the effects of parasites on host behavior. The relationship between *S. occidentalis* and *I. pacificus* is also important for public health, as lizards are hosts for sub-adult ticks, yet block the spread of the Lyme bacterium, limiting Lyme disease[16].

Objective(s): 1) We plan to exploit the natural east-west gradient in tick abundance within SLO County to investigate the effect that the presence of ticks at a particular location has on local western fence lizard behavior, particularly male aggression during the mating season. We will explore the role that host-parasite coevolution has had on shaping the behavior and physiology of these two *S. occidentalis* groups. We will also address assessment capacity in these lizards. 2) This research will require the combined efforts of myself, tenured faculty, and undergraduates. Eleven undergraduates in Dr. Kolluru's and Dr. Vredevoe's labs have already begun assisting with this project, and more will follow. In addition to the primary objectives, methodology, and timeline, the resources obtained for this project can be used to support the independent initiatives of undergraduate researchers on closely related topics, supervised by me, Dr. Kolluru, and Dr. Vredevoe. Future graduate students will also benefit from the resources and methods we develop for this project.

IV. Methodology:

Adult male *S. occidentalis* will be collected from coastal, tick-abundant locales (Poly Canyon) and inland tick-poor locales (Carrizo Plain National Monument, CDFW SC permit and IACUC approval pending). Lizards will be caught using standard[13] lizard "nooses" and by hand. We will measure snout-vent length (SVL) and place males into categories based on ventral blue coverage and intensity. Males will be paired based 1) on SVL (to within 3 mm[1]) and 2) color (as subjectively assessed by blue badge size and saturation). Only males from the same location will be paired (i.e., no coastal male will be paired with an inland male). Pairing will take place at the site of collection, and only paired lizards will be brought back to Cal Poly. We will mark lizards to track them throughout the project, and also to prevent the recapture of rejects. We estimate that approximately 50 pairs (100 males total, 50 from each population) will be required. Male lizards will be housed in overlapping cohorts of approximately 32 individuals. Males brought to Cal Poly will be placed in IACUC-approved laboratory cages in Dr. Kolluru's lab for approximately two weeks to allow ectoparasites (ticks and mites) to reach repletion and be collected. Once clear of wild ectoparasites, one lizard from each pair will be re-infested by cohabitation in a small terrarium with 50 lab-reared larval *Ixodes pacificus* ticks over a period of 48 hours. Lizards will then be housed in enclosures fabricated from plastic pools on the roof of Science North (Bldg. 53). The 32 members of each lizard cohort will be housed in 4 pools subdivided into 8 sections, so that no male lizards interact before trials. At this point, blood may be drawn to determine hematocrit and endoparasite infection status.

Following the general methods of Garland[1] and Huyghe[6], trials will be staged in a 50 x 120 cm arena constructed inside a cooled chamber (possibly a freezer-less refrigerator lying horizontally) at 20^{o}C. The arena will have a sandy substrate with bark hides/basking perches. Paired lizards will be placed at opposite ends of the arena with an opaque divider between. Two 100W heat lamps will initially provide the lizards with individual basking areas. After 30 minutes, the two 100W heat lamps will be switched off, the middle partition removed, and

a single heat lamp switched on in the center of the arena. Lizards will compete for access to the central basking spot. We will record the aggressive (push-up, gular flutter, rush, bite) and submissive (retreat, flatten, hide under substrate) behaviors, the duration of conflict, and winner/loser status, by observing from behind a blind. We will also video record trials.

V. Timeline: 1) January 28, 2016 CDFW permit application mailed. 2) February 2016: Tick rearing 4) Mid-Late April, 2016: 12 weeks from CDFW permit application, taken as starting date for lizard collection at CP and Carrizo. Assuming 32 lizards (18 pairs) per cohort:

a. Lizards captured from coastal and inland locations: 2 days
b. Ectoparasites allowed to feed to repletion and fall off in cages: 10-14 days.
c. Lizards allowed to recover on roof of 53: 1 week.
d. Lizards placed with larval ticks: 2 days
e. Lizards returned to roof: 3 days rest.
f. Trials commence. 3 trials per day: 1 week. Blood drawn again, lizards sacrificed.

Total: 5 weeks. Because cohorts can overlap by two weeks, total time from collection to completion of trials is 11 weeks. 6) Mid July, 2016: Trials complete. Data analysis begins. 7) August-September 2016: Analysis 8) October 2016-January 2017: Write up: thesis, manuscripts, and undergraduate projects. 9) February 2017: Submit to Journals, conferences.

VI. Final Products and Dissemination: The final product of this project is primarily a dataset that can be used in my thesis and multiple students' senior projects. My thesis will be written in a way that will make it easily modified for publication in journals such as *Oecologia*, *Experimental Zoology* and *Parasitology Research*. We also plan to present this work at conferences such as the 2017 meetings for the American Society of Parasitologists (San Antonio, TX) and the Animal Behavior Society Conference (Toronto, Canada).

VII. Budget Justification: Travel to and from the Carrizo plain will be in privately owned vehicles capable of 25 mpg. Assuming a round trip from Cal Poly to the Carrizo Plain and back of 140 miles, $2.70 per gallon, 20 vehicle-trips (e.g. 5 vehicles participating in 4 trips), gasoline for this project will cost $302. Although some other travel within North America for conference attendance may be required, those costs cannot yet be estimated, and so they are omitted from this proposal.

Operating expense: 8' folding pools cost $24 each, but must be modified for use on the roof of Science North. Per pool, modifications include: support by wooden dowels ($10); wood mulch ($16); 8' by 8' plywood base ($20); and 9 cinderblocks ($9). This comes to $79 per pool, or $316 for four. A cooled chamber (such as a low-powered refrigerator) will cost $450. 3 heat lamp bulbs will cost $24. We will be able to provide the other materials called for in the experimental design. The total for this section is $790.

The total funding requested is therefore $1092.

Literature Cited: [1]Garland T, et al. 1990. *Funct. Ecol.* 4(2): 243-250. [2]Schall, J & M Dearing. 1987. *Oecologia.* 73: 389-392. [3]Dunlap, K. & T Mathies. 1993. *Copeia*. 1993: 1045-1048. [4]Bull, C & D Burzacott. 1993. *Oecologia*. 96(3): 415-419. [5]Rutte C, et al. 2006. *Trends Ecol. Evol.* 21(1): 18-21. [6]Huyghe K, et al. 2005. *Funct. Ecol.* 19: 800-807. [7]Lane, R & J Loye. 1989. *J. Med. Entomol.* 26(4): 272-278. [8]Pollock N, et al. 2011. *J. Exp. Zool.* 317A:447-454. [9]Eisen L & R Eisen. 1999. *Exp. Appl. Acarol.* 23: 731-740. [10]Rodgers S, et al. 2007. *J. Med. Entomol.* 44(2): 372-375. [11]Schall, J & P Houle. 1992. *J. Herpetol.* 26(1): 74-76. [12]Dargent F, et al. 2015. *J. Evol. Bio.* 29(2016): 23-34. [13]Schall J, et al. 2000. *J. Herpetol.* 34(1): 160-163. [14]Koskimäki J, et al. 2004. *Behav. Ecol.* 15(1): 169-173. [15]Kura T. 1999. *J. Theor. Biol.* 198: 395-404. [16]Salkeld, D & R Lane. 2010. *Ecology.* 91(1): 293-298.

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CAL POLY

PROPOSAL BUDGET

Student Applicant(s): **Dylan M Lanser**	
Faculty Advisor: Gita Kolluru, **Larisa Vredevoe**	
Project Title: Effects of tick parasitism on aggressive behavior in male western fence lizards: geographic differences in self-assessment capacity?	**Requested Endowment Funding**
Travel *subtotal*	**$302**
Travel: In-state	**$302**
Travel: Out-of-state	**$**
Travel: International	**$**
Operating Expenses *subtotal*	**$790**
Non-computer Supplies & Materials	**$790**
Computer Supplies & Materials	**$**
Software/Software Licenses	**$**
Printing/Duplication	**$**
Postage/Shipping	**$**
Registration	**$**
Membership Dues & Subscriptions	**$**
Multimedia Services	**$**
Advertising	**$**
Journal Publication Costs	**$**
Contractual Services *subtotal*	**$0**
Contracted Services	**$**
Equipment Rental/Lease Agreements	**$**
Service/Maintenance Agreements	**$**
TOTAL	**$1092**

CAL POLY
Animal Science Department
1 Grand Avenue, 010-0147
San Luis Obispo, CA 93407-0255

01-Feb-2015

Michael D. Miller, PhD
Baker and Koob Endowments Coordinator
Executive Vice President for Academic Affairs
California Polytechnic State University
1 Grand Avenue
San Luis Obispo, CA 93407

Dr. Miller:

This recommendation is on behalf of **Breanna Modica** and her proposal submitted for the annual **Baker and Koob Endowments** from **California Polytechnic State University**. I have had the personal privilege of working with Breanna, as her academic advisor, instructor and supervisor, during her undergraduate studies in Animal Science and her graduate committee chair at California Polytechnic State University. Ms. Modica is an exceptional student. A transfer student into our program, she successfully completed our rigorous undergraduate science curriculum with 3.389/4.0 GPA. Additionally, her academic performance was recognized twice on the college Dean's List. Upon completion of the first year of her graduate level courses, she currently maintains a 3.568/4.0 GPA.

Ms. Modica's thesis research is related to the influence of fiber length in leopard tortoises, our research model for grazing herbivorous reptiles with adaptations for postgastric, microbial fermentation. This is an animal health issue of particular interest, as herbivorous reptiles maintained in managed environments are often fed diets lower in fiber than natural diets and/or diets with highly processed fiber particles that may not result in similar fermentation patterns in the hindgut.

As Breanna prepared her review of relevant literature, as well as participating in an international Comparative Animal Nutrition symposium, she began to consider how the microbiome of the gastrointestinal tract may be influenced by dietary substrates differing in fiber length, but not nutrient content. She proactively sought collaborators on-campus across colleges that could advise her in appropriate methodology, as well as data analysis and interpretation. As of this date, all of the necessary samples have been collected and are available for immediate analysis as resources allow.

Breanna identified, applied for and successfully secured partial funding for this phase of her work through a competitive **Herpetological Grant** generously offered by **East Texas Herpetological Society**. The support requested from the **Baker and Koob Endowments** represents the necessary balance to complete sample and data analysis.

Breanna is coordinating all aspects of this project, including formulation and manufacturing of test diets, management of animals, coordinating undergraduate labor, and developing analytical procedures. Quite frankly, without her efforts, our current scholarly work on fiber utilization in herbivorous animals, and the broad undergraduate involvement associated with that work, would not occur. She demonstrates creative problem-solving skills and resourcefulness to accomplish the work required, often with little or no direction from myself.

Simply, Breanna continues to exceed my expectations, not only in the amount of work and impact she has with our students, but the exceptional quality of that work and those interactions. She demonstrates a level of maturity not observed among her peers, which I believe is exemplified by her application for this grant.

In a short time, she has become an important asset to our department and undergraduate students. I am committed to investing in Ms. Modica as a part of her preparation for a career in comparative animal nutrition, and I would strongly advocate her work is worthy of your investment as well.

I am confident Breanna would not only greatly benefit from your support, but she would represent the award with a positive attitude and personal responsibility. **Breanna has earned my highest recommendation.** If you have any additional questions or would like to discuss Breanna further, please contact me at msedward@calpoly.edu or 805.756.2599.

Sincerely,

Mark S. Edwards, Ph.D.
Professor, Comparative Animal Nutrition
Associate Department Head